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THE BIOLOGY OF DIPHTHERIA.

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The Biology of Diphtheria.

No other infectious disease has been so extensively studied as diphtheria, and no disease has presented such interesting phenomina in the field of bacteriology and immunology. These interesting phenomina have stimulated the efforts of numerous investigators and resulted in the accumulation of a vast amount of material on the subject, but in spite of the many interesting principles revealed no one has yet found a way of controlling, even in civilized countries, the prevalence of the disease.

The nature of the disease. Diphtheria is transmitted by the passage of the Klebs-Loeffler bacillus, the infective agent, from one person to another. The organism passes out of the mouth or nose of sick persons, or in some case healthy "carriers" of the germs, and lodges in the throat of other persons. Here it growns locally, producing a lesion known as a false membrane, and generating a portion spoken of as diphtheria toxin. The latter passes from the lesion into the circulation and produces the general symptoms of the disease. The bacteria themselves do not ordinarily get beyond the local lesion, but the toxin which they produce and which is distinct from their own body cells, is widely disseminated.

History. In ancient times diphtheria appears to have prevailed in certain countries and to have been absent in others. It was well known in Egypt, Palestine, and Syria. In the writings of the physicians of Hippocrates' time there is no description of it and the assumption is therefore made that diphtheria did not exist in ancient Greece.

Epidemics spread over parts of Europe at various times during the Middle Ages, as indicated by the numerous descriptions of throat pestilences. A large epidemic started in Spain in the second half of the 16th century. In the early part of the 18th century the disease spread over the whole of Europe and into America, but indications are that in areas not attacked by epidemics the disease was extremely rare. In 1830 a pandemic spread over a large part of the world having its height in 1850 and 1860. Since then the disease has been endemic in nearly all civilized countries, a condition of marked contrast to the irregular occurrence at early periods.

Early opinions concerning the nature of the disease. The first accurate account of diphtheria was given by Aretaeus in Rome at the end of the first century (1)# but it was not until 1765 that Home (2) gave the first accurate clinical description and aroused the interest of the world. Brettaneau in 1826 (3) studied several outbreaks and was first to recognize that various forms of the affection, such as malignant angina and croup, were a part of the same disease entity. A controversy existed for many years as to whether the local throat condition was due to the entrance of an infective agent. Sachse in 1810 (4) states that, at that time, opinions were almost equally divided. Brettaneau held strongly to the infection theory and introduced much evidence to support his views. Many experiments were performed in attempts to inoculate persons and animals with fragments of tissue from diseased persons. Attempts were made to produce the local throat condition by acrid chemical substances, and Bretteneau himself produced a false membrane in the trachea of dogs with cantharides and olive oil. A few of the animal inoculation experiments were successful (5,6) but none threw appreciable light on the nature of the disease.

Numbers in parenthesis refer to references at end of paper.

Discovery of the diphtheria bacillus. The revelation of the cause of the disease followed the development of the science of bacteriology. Bassi's discovery of the fungus nature of muscardine a silk worm disease, lead to the doctrine of "living contagion" and many attempts followed to find a similar fungus in the lesions of persons infected with diphtheria. The next forty years ~~is~~ are filled with innumerable claims for discovery of the particular microbe causing diptheria, but it was not until 1884 that Loeffler (7) found a bacillus,with the help of the methylene blue stain which he devised, which satisfied Koch's postulates.*

Discovery of Diphtheria toxin. Loeffler noted that animals injected with his diphtheria bacilli sometimes died without appreciable bacterial invasion of other organs of the body. From this he concluded that death was brought about from the action of the chemical poison produced at the site of inoculation and absorbed into the body. In 1887 he isolated from the culture medium of a culture of diphtheria bacilli a substance which was very toxic when injected into guinea pigs (8).

The credit of the discovery, however, is usually given to Roux and Yersin (9) who used somewhat different methods. They grew the bacilli on veal broth and filtered the culture through porcelain candles and obtained a toxin which was much stronger than that obtained by Loeffler.

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* "Koch had shown that his three postulates must be fulfilled before the relation of a micro-organism to a disease could be proved. Firstly, the organisms must always be found arranged in a characteristic manner in the affected part; secondly, these organisms must be isolated and obtained in pure culture; thirdly, it must be possible to reproduce experimentally the disease by the inoculation of pure cultures."

of pure cultures."

The discovery of diphtheria toxin marked a great advance in understanding the nature of the disease. Th idea of toxin absorption from the local lesion reawakened the idea of the application of local antiseptics to the throat lesions, and Loeffler in 1891 (10) claims to have had good results with a mixture of toluol, alcohol, and iron chloride.

Discovery of diphtheria antitoxin. In 1890 - 1892 Behring, Kitasato, and Wernicke (11) published their work on immunity to diphtheria and tetanus, a discovery which marked one of the greatest achievements in medicine. They showed that animals injected with small quantities of diphtheria toxin acquired an immunity both to the bacteria and the toxin. They showed that this resistance was due to antitoxins, substances circulating in the blood of the treated animal, and that, if this blood were injected into another animal, it gave protection to the second animal. An animal could not only be protected from acquiring the disease by the injection of this immune blood, but could in some cases even be cured after the disease had set in.

This step represents a great contribution to our knowledge of immunity in general. Before that time vague theories had been expressed to explain the immunity conferred by an attack of a disease. Pasteur had held a view known as the "exhaustion theory" that, during an infection, specific nutritive substances necessary for the growth of the particular organism were used up, so that if the body became subsequently infected with the same organism this organism would not find suitable material for its growth. Others thought that protection was afforded by the development of substances in the body unfavorable for growth of the organism, a view known as the "retention theory" of Nencki, Chaveau, and others. Behring formulated a theory based on the alkalinity of the blood and Baumgarten conceived the idea of harmful osmotic conditions. Finally Metchnikoff was championing the idea of the protective action

action of the leukocytes, "phagocytic theory", while the Flügge School was championing the idea of bactericidal properties of the blood serum.

The discovery of antitoxin was, of course, a great victory for the Flügge School, but, as will be seen later, it was soon found that this mechainism was one that functioned in only a small number of other infectious diseases.

Tetanus toxin was discovered in 1891 (12) and toxins of a few other bacterial diseases were demonstrated in the following years. Ehrlich and others worked out an analogous antibody production for certain non-bacterial substances, namely, several plant poisons, snake venoms, and certain digestive ferments.

The nature of toxins. Much interest was taken in the chemical nature of these poisons. Their susceptibility to heat, deterioration on standing, and antibody stimulation suggested a close analogy to enzymes. However, all attempts at isolation of the toxin from the protein molocules of the culture broth failed, and investigators were forced to learn what they could from biological studies.

One of the peculiar properties of toxins is the delay that takes place between the injection into an animal and the development of symptoms. If tetanus toxin is injected into mice symptoms never develope before eight to nine hours no matter how large the dose.(13), and if diphtheria toxin is injected into a susceptible animal no reaction can be obtained under eight to twelve hours even when thousands of lethal doses are given. Erlich explains this as indicating a delay between the union of toxin with the cells and the subsequent injury; Richet,considered that toxin is not potent until acted on by the body; and DeWaele considers that there is a gradual concentration of toxin into the vulnerable tissues.

Tissues susceptible to toxin. Diphtheria and tetanus toxins are never absorbed through uninjured mucous membranes in spite of their extreme toxicity (14,15,16,17). Toxic substances are not absorbed from the alimentary canal from cholera vibrios until the intestinal wall is injured (18,19).*

When circulating in the body diphtheria toxin shows a remarkable selective action on certain tissue. Concentrated amounts cause necrosis in nearly all tissues, the process being very like that found in the false membrane of diseased throats and in the subcutaneous tissue at the site of injection into animals. Small amounts in the general circulation seem to have a special affinity for the nervous system, but affect~~s~~ also the heart, kidneys, spleen, and adrenals. Meyer and Ransom (21) have shown that diphtheria toxin may be absorbed through the nerve endings locally in the same way as tetanus toxin (22). This action is believed to be due to a chemical or physical affinity of the toxin for the particular type of cell, a process which is analogous to the affinity of narcotic drugs for the nervous tissue, and of bacterial dyes for particular cellular elements.

A note worthy fact in this connection is the disappearance of toxin in the circulation of susceptible and non-susceptible animals. When injected intravenously into susceptible animals a large amount quickly disappears (23) and is not present in the urine, but if injected into ~~the~~ non-susceptible animals, such as the turtle, it persists for a long time (Metchnikoff). These facts may be taken to indicate that susceptible animals bind toxin to their tissues while others allow it to remain in the circulation.

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* Botulism toxin forms an exception to this rule, but it must be remembered that this toxin is formed not as a product of bacterial metabolism but as the result of the action of bacterial products on decomposing food stuffs; bacterial products are not absorbed.(20).

Rats are very resistant to injections of diphtheria toxins and can stand 1500 (24) to 3500 times as much per body weight as guinea pigs. They are quite sensitive to intracerebral injections, however, a fact which has led to much controversy as to how subcutaneous injections are prevented from reaching the brain. That the toxin is not neutralized and anchored by natural antitoxin present in the tissues is shown by the fact that the rat does not possess any natural antitoxin (25). Its immunity, therefore, depends upon a non-susceptibility of its tissues to the action of the toxin. Metchnikoff claims that leukocytes set up a barrier around the sensitive cells and protect the toxin from reaching them.

Wasserman and Takaki (26) showed that brain tissues mixed with tetanus toxin neutralized its harmful effects, a reaction which was subsequently shown to be equivalent to neutralization by antitoxin (27). Metchnikoff (28) moreover showed that the neutralizing power of brains of various animals was proportionate to their susceptibility to the poison.

The work of a number of observers has shown the likelihood of the lipoid substances as being the element which brings about union with the poison, but Bang points out the possibility that the lipoid mechanism may be only an indirect one by an alteration of the solubility of the tissue elements.

Reaction between toxin and antitoxin. After the discovery of antitoxin discussion arose as to the nature of its protective action. First it was thought that antitoxin destroyed toxin; later, that antitoxin acted on the body cells giving them protection. In 1894 Calmette and others (29) working with snake venom and its antitoxin showed that this poison was much more resistant to heat than its antitoxin. If the two were put together in proper amounts the mixture was harmless, but if the mixture were first heated beyond the antitoxin death point it was made toxic again, a fact which showed that the antitoxin or antitoxic portion of the complex was inactivated and dissociated

from the toxin. Morgenroth (30) dissociated the mixture in a similar way with hydrochloric acid. This evidence indicated that the toxin-antitoxin complex can be dissociated.

Ehrlich (31) found that the toxin and antitoxin did not combine in regular proportions, but that the addition of small amounts of antitoxin did not always neutralize an equivalent amount of toxin. This led him to believe that the combination was in the nature of that of two chemical substances, and that antitoxin had a binding power for toxin analogous to the valency of one element toward another. His experiments led him to believe that antitoxin had a valency of 200 as compared to toxin, or "Toxin$_{200}$Antitoxin". His theory is based largely on a conception of toxons and toxoids, substances present in filtrates having a somewhat different binding power than toxin.

Ehrlich noted that it took only a relatively small amount of antitoxin to protect a guinea pig from death by a large amount of toxin, but that it took an equivalent amount to protect from sickness in both large and small amounts of mixtures. Large amounts of toxin could thus be partially neutralized by a relatively small amount of antitoxin and the resultant mixture would give rise to chronic illness, but not death. With small amounts of mixtures the animal was either killed or unaffected. Ehrlich explained this phenomina on the basis of "toxons", additional toxic substances in the filtrate which in large quantities caused the above mentioned chronic illness, but which, if sufficient antitoxin were added, were neutralized in the same way as toxin. Toxons, he

assumed, had less affinity for antitoxin than toxin itself, so that toxin would always combine with antitoxin first. In small quantities of mixtures, then, it was a matter of neutralizing toxin only, as too few toxons were present to have effect. Large quantities might have all the toxins neutralized but have large amounts of toxons free.

Arrhenius and Madsen (32) explained this phenomina as being analogous to a reversable chemical reaction. Bordet (33) assumes the explanation to be a process of partial saturation of the toxin by antitoxin, like the varying amounts of iodine absorbed by starch. Subsequent observations indicate that the combination is much more complex than was originally antiscipated. Danz (34) showed that neutralization depended on the manner in which the toxin and antitoxin were added together. Park and Atkinson (35) showed that three-fold neutralized toxin stimulated antitoxin formation in horses and Pritchett (36) produced thickening of glomerular capsules of guinea pigs by injecting mixtures which produced no skin lesions. However, the strict specificity of the reaction as originally observed by Behring has been found to hold true in all cases; in other words, the antitoxin of one poison has no effect on any but its own toxin. That antitoxins from all strains of diphtheria bacilli are not exactly identical is indicated by recent work done by Havens (37) in which it was shown that all the virulent diphtheria bacilli fell into two groups according to agglutination reactions and that the antitoxins formed by members of one group were not exactly

identical. His work indicates the possibility of enhancing the value of commercial antitoxin by the addition of antitoxin prepared from a member of the second strain of bacilli.

Antitoxin in human blood. Abel (38), as early as 1894 showed that antitoxin was present in the blood of many normal persons. Schick devised a simple skin test which consisted of the injection of a minute quantity of toxin between the layers of the skin, a test which has been used on a wide scale to determine the presence of natural antitoxin. Since that time there has been no authentic case where a person, showing the presence of antitoxin by this test, has ever subsequently contracted a case of diphtheria(39).

The *uses* of *antitoxins*. Commercial antitoxins, consisting of the blood serum of immunized horses, was put on the market for the first time in Höchst, Germany, in 1893. As the therapeudics effects of antitoxin are well known to everybody, the matter need not be taken up here. The prophylactic value of the serum should not be forgotten, however, as its injection renders a certain and prompt method of protecting all persons exposed to the disease. This prophylactic injection of antitoxin is spoken of as passive immunization.

Active immunization may be brought about in persons by a method similar to that employed for obtaining serum in horses. Toxin injections in small doses is difficult to control, however, and in place of it a mixture of toxin and antitoxin is used in which some free toxons are present (40). These stimalate antitoxin formation in favorable cases.

Active immunization is not always successful, and the response is often delayed for several weeks after injection. The immunity lasts much longer than that from the passive injection of serum, and Park believes that it may be possible to immunize children throughout life (41). A number of children have been treated in institutions in New York City and have shown by the Schick test persistence of antitoxin over a number of years. This, Park claims, is an indication that antitoxin production can be aroused (in children at least) and made to function as a continuous physiological process throughout life. Natural antitoxin production is thought by some to be formed in a similar way in response to stimuli from small "subinfective" doses of diphtheria bacilli with their toxin.

Summary of toxin-antitoxin concepts. To recapitulate it may be stated that diphtheria is to be regarded as a local infection, the organism entering the upper respiratory passages, growing there and producing local lesions from which the poison excreted is absorbed into general circulation. The impression gained is doubtless that, since the poison represents the only direct causative factor in the disease, resistance to infection depends entirely on the presence or absence of antitoxin.

As to the certainty of the protection afforded by antitoxin there can be no doubt, though a few observers state that considerably more antitoxin may be needed under special conditions, such as when suffering from scarlet fever. However, there is much evidence to show that absence of antitoxin is not always accompanied by a high degree of susceptibility.

This is indicated by the following facts:

1. Persons in intimate contact with cases of diphtheria and not possessing natural antitoxin are infected in only a small percentage of cases.

2. Healthy persons frequently harbor virulent bacilli in their throats and show no manifestations of the disease even when they possess no antitoxin.

3. Persons frequently recover from cases of diphtheria without acquiring antitoxin(42).

This evidence is certainly highly suggestive that other protective mechinisms may be called into play in cases where antitoxin is absent.*

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* The assumption is generally made that the natural cure of diphtheria in man takes place as a result of antitoxin formation in response to the stimulating action of the toxin, an action similar to that originally observed by Behring and his collaborators when they discovered antitoxin. That this is frequently not the case has been shown by Park's observations. Recovery without antitoxin has been referred to as being an example of bactericidal immunity, but no work so far has been done to throw light on the nature of the mechanism.

General types of immunity. The discovery of antitoxins in 1890 - 1892 led to attempts to find similar substances in other diseases. Only a few of these attempts were successful. Pfeiffer (43) found that cholera filtrates were Only very slightly toxic, but that the washed and killed bacterial bodies were quite poisonous. He assumed that, in this disease,

the poison was in the bacterial cells, and he spoke of such poisons as "endotoxins". Pfeiffer's conception was that endotoxins were specific preformed substances retained in the cell bodies and liberated only upon the disintergration of the organism. If bacteria were in the system, then, the poison liberated would depend not on the growth of the organism but on the number of cells destroyed. This theory was substantiated by the observation that immune animals, in which organisms were quickly destroyed, were more subject to toxic effects upon injection of dead organisms, than were non-immune animals.

Recently Vaughan (44) has demonstrated the fallacy of the "endotoxin" theory. He has devised a method of obtaining cleavage products from prteins with alkalinized alcohol, and finds that all proteins, bacterial or otherwise, yield "toxic split products" which have all the biological properties of Pfeiffer's "endotoxins". Friedberger has shown that these same toxic split products are formed when either pathogenic or non-pathogenic bacteria are acted upon by blood sera.

These results indicate that in those diseases where no toxin is produced infection does not depend on any specific poison in the bacterial cell, but upon poisonous substances formed when the bacterial bodies are disintegrated in the blood stream. Pathogenicity, then, is not dependent upon a poison producing property of bacteria, but solely upon the power of an organism to invade and establish itself within the body tissues.

Bacterioidal resistance. Pfeiffer was the first to recognize one of the most important mechanisms by which bacterial invasion is resisted. He and Isaeff (45) made a

number of experiments commonly spoken of as the "Pfeiffer Phenomina". They showed that the injection of cholera spirilla into the peritoneal cavity of guinea pigs produced a property in the animals' blood serum capable of dissolving the bodies of the spirilla. This property was, moreover, a specific protection for the bacteria used, the resistance to other bacteria being unaltered. This was the first example of, what Pfeiffer called, "specific bactericidal substances". Examples of other types of immune antibodies were subsequently discovered, ~~mainly~~ namely, agglutimins, precipitins, and opsonins - all specific substances formed in the blood serum upon immunization, but acting on the bacteria by different mechanisms. It must not be concluded, however, that all powers of resistance are represented by specific antibodies to bacterial cells or their toxins. Metchinkoff (46) was the first to recognize the great significance of bacterial destruction in the blood and tissues by means of the engulfment by leukocytes, a process spoken of as phagocytosis. While later work has shown that phagocytosis is largely a "scavenger" process for the removal of dead material, rather than a killing process, and that in many cases destruction is dependent entirely on the presence of other substances known as opsonins; there is still much ground to believe that phagocytosis is also a general protective mechanism. Similarly there is evidence to believe that, in addition to the other specific bactericidal substances mentioned by Pfeiffer, there are general bactericidal fluids in the circulation capable of inhibiting the growth of, or even destroying to a certain extent, all types of organisms.

Biology of the diphtheria bacillus. Before discussing the role played by a possible bactericidal resistance in diphtheria it is important to keep in mind the habits of growth of the diphtheria bacillus. As far back as 1888 (47) Roux and Yersin had observed that intravenous injection of diphtheria bacilli into rabbits was not invariably followed by death. Loeffler had made similar observations at an even earlier date. Metin in 1898 (48) had found that, after intravenous injections of the bacilli, very few could be found in the blood after a few hours, but that, if the spleen were incubated after death, large numbers could be found. With subcutaneous injection it was difficult to isolate organisms from the site of injection until some time after the death of the animal. Numerous other experiments might be quoted to show that diphtheria bacilli do not grow readily in living tissues of even those animals which are susceptible to the disease. That leukocytes play a part in inhibiting the growth ha~~ve~~s been shown by the rapidity with which bacilli are phagocyted when injected intraperitoneally. Wright (49) has shown that phagocytosis of diphtheria bacilli is not influenced by opsonins, but Tunnicliff (49a) claims that opsonins do play a part.

A study of pathological lesions of the disease furnishes even more interesting information concerning the habits of growth of the organism. The common throat lesion, which is spoken of as a false membrane consists of a layer necrotic tissue containing fibrinous exudate. Diphtheria bacilli are found in large numbers in all parts of the necrotic tissues, but are almost invariably absent in living tissue. This indicates that the bacteria do not possess, of themselves, the power to invade, but that they follow the path of necrotic

tissue prepared for them by their toxin. "It seems probable that the beginning of the lesion is due to the toxic action of bacilli growing in the fluids of the mouth or throat. When necrosis is once produced the necrotic tissue and the membrane deposited on its surface form a suitable culture medium."(50)

In a certain proportion of cases, especially severe and fatal cases, bacteria get into the circulation, infect the servical lymph nodes, and in rare cases even produce general septicaemia (51). These cases represent, in all probability, a condition of such lowered resistance on the part of the host that they need not be considered as having significant bearing in the matter of immunity. To recapitulate it may be stated that the diphtheria bacillus is essentially a saphrophytic organism, growing on dead organic matter, depending for its pathogenic power solely on the aggresive action of its toxin.

Sources of infection. Besides in actual cases of diphtheria the bacilli are frequently found in the throats of other persons. These persons are spoken of as diphtheria "carriers", and represent one of the common sources of infection. Williams classifies these persons into three groups according to whether the carrier has (1) general symptoms, such as anaemia and increased pulse rate, together with local symptoms such as nasal catarrh or pharyngitis; (2) local symptoms alone; or (3) no symptoms. In addition to these groups there are convalescent carriers, persons who harbor the organisme in their secretions

for an indefinitely prolonged period after recovery from diphtheria. Persons harboring diphtheria bacilli who have not been in contact with known carriers or cases are rarely found (52), and it can reasonably be assumed that human sources of infection never arise de novo, but only result from contact with cases or other persons harboring bacilli acquired from contact with cases.

The persistence of organisms in the discharges of convalescents long after their recovery is dependent in nearly all cases on a diseased condition of the throat or nasal passages. Healthy secretions do not seem to be a suitable culture medium for diphtheria bacilli. In prolonged cases the infection seems to persist in pockets and folds of tonsils, sinuses, or diseased portions of mucous membranes. The occasional persistence of organisms in these cases, in spite of all attempts to remove them,by antiseptics, agglutinating serums (53), ferments, and cultures, and the prompt clearing up of the infection when all diseased conditions have been removed (54), indicates that conditions in the normal passages are not suitable for the contiuous growth of the bacilli.

Diphtheria has been observed in a number of domestic animals. Dogs, guinea pigs, and rabbits are quite susceptible but have never been found infected in nature. There is some evidence that cats contract diphtheria, and an example is quoted by Low 1898 in which a cat contracted diphtheria from a human case which infected another cat which subsequently infected four children. Simmons (55) quotes another example in which an old lady was infected from a cat. Klein (56)

infected cows which developed ulcers on their teats containing bacterial bacilli. Some authors have shown that ulcers on the teats of cows may become infected with ~~bacterial~~ diphtheria bacilli and thus infect milk. This would seem a logical explanation for the source of many epidemics described in the literature, but in most cases a study of these epidemics has shown that the source of infection was superadded to the milk in the process of handling. True diphtheria is a very rare disease in horses. Cobbett (57) quotes and example of a child being infected from a pony. ~~and~~ Baulinus (58) describes examples of diphtheria contracted from birds, but most observers consider that the"diphtheritis" of birds is a different disease from human diphtheria. Considering the relative infrequence of animal infection as compared to human, the former can be regarded for practical purposes as being of insignificance importance as a causative factor in the spread of the disease.

Modes of transmission. The passage of organisms from throat to throat may take place by the inhalation of organisms suspended in the atmosphere as suggested by Welch in 1891 (59), but Wright and Emerson (60) and Sudeck (61) could find no diphtheria bacilli in the air of hospital wards. Diphtheria bacilli can be easily found on articles in sick rooms,indicating that indirect transmission can be a means of spread. Chapin (62), however, claims that this means is much rarer than that of direct contact.

In rare cases bacilli remain dormant for long periods in a dried state and subsequently give rise to infection. Bugbee (63) described an interesting example of

infection from a book stored eleven months. In a few cases bacteria have been said to survive long periods in dust or hair, but in most cases cited the survival seems to depend on the protection afforded by dried secretions.

Factors involved in infection. As mentioned in a previous paragraph, persons devoid of natural antitoxin when exposed to diphtheria contract the disease in only a small proportion of cases. What, then, are some of the factors that determine these chances of infection?

The contention is sometimes made that infection depends largely on the quantity of material in the infecting dose. There is some just ground for this contention in view of the numerous cases cited in the literature in which accidental infection by large amounts of infective material, such as pieces of membrane (64), have resulted in very severe infections. In these cases it is quite natural to suppose that the infective material carries with it enough preformed toxin to injure a small area of mucous membrane and thus prepare suitable material for the establishment of the infection. Cases of this sort should be considered the exception and it is very doubtful if a similar mechanism functions in ordinary infections.

These facts might indicate that bactericidal properties play no part as a means of protection. It is quite possible, however, that even if the number of bacteria are not kept down the invasion of the healthy tissue by them is prevented. Therefore, it might be well to bear in mind the possibility of protective porperties of the secretions (65,66),

and ideas such as are suggested by (1) ~~the ideas of~~ Metchnikoff concerning the protective properties of the lymphoid tissues, (2) the demonstration of the wandering of the leukocytes from the tonsils (67), and (3) the observations of numerous later investigators of the activity of the phagocytes of the respiratory passages. Whatever parts may be played by leukocytes it is indicated that specific opsonins do not play a part, but that the leukocytes act only as general scavengers.

As to whether toxins are formed in the secretions is doubtful, as no work as been done on this subject; but in view of the sensitiveness of toxin production to the reaction of the culture medium, (68) and to its chemical constituents (69), it would seem quite unlikely that secretions would be suitable, in more than a small proportion of cases, for massive toxin production. If much toxin is formed it is likely that some of it would come in contact with the mucosa in sufficient concentration to initiate a lesion.

The barrier against this initial lesion, where one does exist, must be due either to processes hitherto unknown, to mechanical factors such as the shedding of epithelium, cleansing action of secretions, and mechanical removal (70), or combinations of many different factors already referred to in a manner which may be spoken of, for want of a better word, as "chance".

Whatever are the exact protective mechanisms, the present state of our knowledge demonstrates that, in addition to the great benefits of specific therapy and immunization, one of the greastest factors in the spread of the disease is

the great reservoir of infection in the carrier state, the majority of which owes its persistence to the general prevalence of diseased or semi-diseased conditions of the noses and throat of the general population.

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